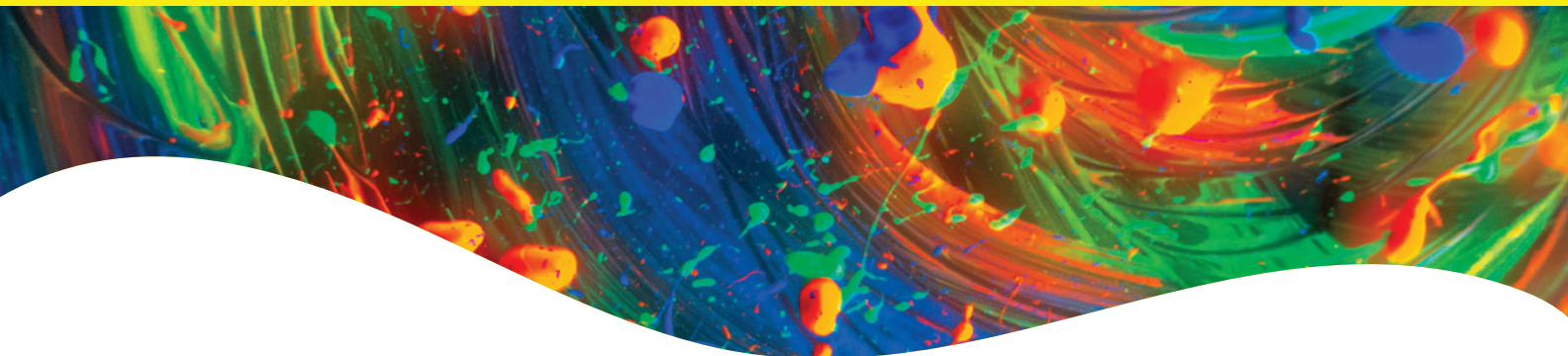




PhenoVue 505 Live Cell Caspase-3/7 Activity Stain



Overview

PhenoVue™ 505 live cell caspase-3/7 activity stain is a no wash, cell permeable fluorogenic dye which is specifically cleaved by activated pro apoptotic caspases 3 and 7 in living cells, leading to fluorescence increase.

Sensitive, rapid and photostable, PhenoVue 505 live cell caspase-3/7 activity stain exhibits green emission and can be multiplexed with blue, orange and red colors. PhenoVue 505 live cell caspase-3/7 activity stain uses a DEVD substrate conjugated with a non-fluorescent cell permeable DNA binding dye which becomes fluorescent when cells undergo apoptosis.

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue 505 live cell caspase-3/7 activity stain	CP22G1	1	100 μ L (1 mM DMSO)	Liquid	Dry ice

Storage and stability

- Store stock solution at -16 °C or below, protected from light. Avoid repeated freeze / thaw cycles.
- The stability of this product is guaranteed until the expiration date provided in the Certificate of Analysis, when stored as recommended and protected from light.
- Centrifuge vial briefly to collect contents at bottom of vial before opening.

Equivalent number of microplates

Product name	When used at concentration	96-well microplate (100 μ L - 300 μ L per well)	384-well microplate (25 μ L - 90 μ L per well)	1536-well microplate (4 μ L - 12 μ L per well)
PhenoVue 505 live cell caspase-3/7 activity stain	5 μ M	0.5 to 2	0.5 to 2	1 to 3

View our full range of high-quality imaging microplates at [Revvity.com](https://www.revvity.com)

Recommended reconstitution

Product name	Stock concentration	Working concentration range*
PhenoVue 505 live cell caspase-3/7 activity stain	Provided as 1 mM in DMSO	Start with 5 μ M Optimization range: 1 – 10 μ M

* Dilutions can be done in cell culture medium or PBS +/- 5% FBS.

Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)	Common filter set
PhenoVue 505 live cell caspase-3/7 activity stain	505*	530*	FITC

* in the presence of dsDNA.

Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixed-cell staining
PhenoVue 505 live cell caspase-3/7 activity stain	Yes	No

Note: PhenoVue 505 Live cell caspase-3/7 activity stain is cell permeable and requires caspase 3/7 enzymatic activity to turn fluorescence on. Therefore, this dye is compatible with live cells but cannot be used with fixed samples.

Protocols

Cell culture

Seed cells in imaging microplates (or any other convenient cell culture vessels). Incubate in the appropriate cell culture conditions, usually 37 °C, 5% CO₂ until 50-70% confluency.

Note: PhenoVue 505 live cell caspase-3/7 activity stain is compatible with live cells only and cannot be used with fixed samples.

Cells can be treated with compounds of interest either before (endpoint) or after (timelapse acquisition) PhenoVue 505 live cell caspase-3/7 activity stain incubation. Staurosporine can be used as positive control to induce cell apoptosis (1 μ M \geq 4h).

Live-cell imaging

1. Remove cell culture medium.
2. Incubate with 1-10 μ M of PhenoVue 505 live cell caspase-3/7 activity stain in cell culture medium (or PBS +/- 5% FBS possible for short incubation time) for 30 min or more at 37 °C, 5% CO₂.

A cell permeable nuclear staining dye, such as PhenoVue Hoechst 33342, can be incubated prior to PhenoVue 505 live cell caspase-3/7 activity stain (before step 1) or just before acquisition. Note that nuclear staining dye could induce cell cytotoxicity and optimal concentration requires optimization.

3. Acquire images on a live-cell imaging device.

Recommendations:

We recommend to not remove the PhenoVue 505 live cell caspase-3/7 activity stain prior to the acquisition (no wash experiment). As the dye is fluorogenic, it displays very low fluorescence background when free in medium or cells. It becomes fluorescent only when cleaved upon activated caspase 3/7 enzymes.

Stain concentration and acquisition settings (excitation power and exposure time) should be optimized depending on the cellular model studied.

This stain can be diluted in cell culture medium (recommended for long incubation time to preserve cell viability) or PBS +/- 5% FBS (for short incubation time).

PhenoVue 505 live cell caspase-3/7 activity stain can support 0.1% Triton X-100 but signal may decrease after permeabilization and washing.

Safety information

Chemical reagents are potentially harmful, please refer to the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Applications

- High-content analysis / high-content screening
- Imaging microscopy
- Live cell imaging

Validation data

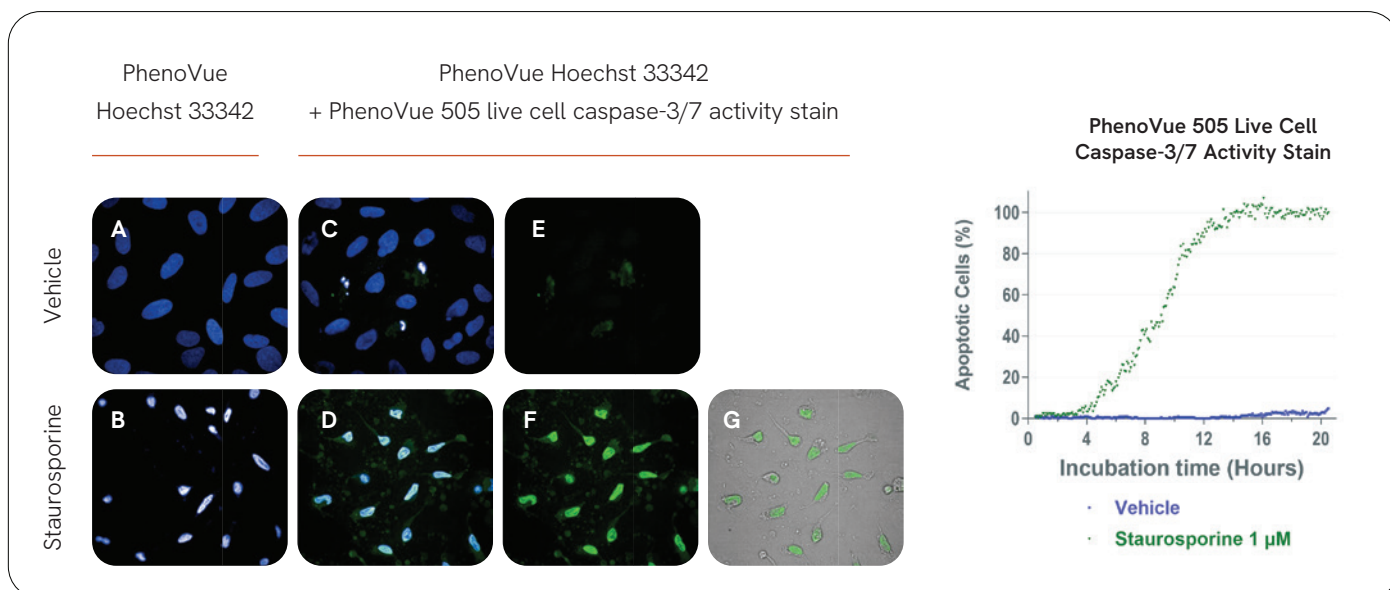


Figure 1: U2OS cells (15,000 cells/well) were seeded in PhenoPlate™ 96-well microplates and incubated at 37 °C, 5% CO₂ for 24 h. Cells were stained with 125 ng/mL PhenoVue Hoechst 33342 for 30 min at 37 °C, 5% CO₂, then the cell culture medium was removed prior to the addition of PhenoVue 505 live cell caspase-3/7 activity stain in the presence of staurosporine (1 μM) for an additional 30 min at 37 °C, 5% CO₂ before time-lapse acquisition. Images were acquired on the Opera Phenix® Plus high-content screening system in time-lapse with 63X water objective, confocal mode (every 5 min for 24h). Images represent 24h incubation time whereas the graph displays the percentage of apoptotic cells (i.e. cells with positive signal of PhenoVue 505 live cell caspase-3/7 activity stain) over time. Panels A and B represent cells stained with PhenoVue Hoechst 33342 only.

Panels C, E, D, F, G represent cells co-stained with PhenoVue Hoechst 33342 and PhenoVue 505 Live cell caspase-3/7 activity stain.

Panels C, D, E, F and G represent fluorescent signal in the PhenoVue 505 Live cell caspase-3/7 activity stain channel (Excitation: 490 nm Laser, Emission: 500-550 nm).

Panels A, B, C and D represent fluorescent signal in the PhenoVue Hoechst 33342 channel.

Panel G represents brightfield channel.

As expected, staurosporine treatment induces apoptotic cell death which is associated with nucleus fragmentation and cell shrinking evidenced by PhenoVue 505 Live cell caspase-3/7 activity stain positive signal compared to the vehicle condition.

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